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Challenge to evaluate regulatory compliance for nutrients in infant formulas with current state-of-the-art analytical reference methods

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ABSTRACT

Infant formulas are strictly regulated and rigorously tested for compliance. Recently, new official analytical methods/standards have been established for nutrient analyses in these product categories through the Stakeholder Panel on Infant Formula and Adult Nutritionals (SPIFAN), governed by AOAC INTERNATIONAL. Many of these methods have been adopted or are in the process of being adopted as reference methods by Codex Alimentarius. The purpose of this paper is to assess the ability of these cutting-edge analytical methods to deliver acceptable results in the context of established regulatory limits for nutrients in food standards and regulations. For this evaluation, the analytical method variability is considered as one of the three main sources of overall process variability, which also includes variation in raw materials/ingredients and the manufacturing process.

The process capability (C_p) is a concept for determining the overall process variability relative to specification limits for a parameter in the final product. Based on this principle an analytical method capability (C_m) was defined and calculated for SPIFAN methods. Global regulatory requirements were evaluated including minimum and maximum limits and tolerances from the declared label values. Compared to these requirements, analytical methods for vitamins A, B_{12}, D and folic acid are of particular concern in relation to the requirements in China,

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some EU member states, Pakistan, Russia, Singapore, South Africa and Thailand. For a product with a manufacturing target at the midpoint of the regulatory range for these nutrients, the probability of obtaining an analytical result outside the regulatory requirements due to analytical variation alone can be as high as 19%. This does not consider variability caused by the production process and/or raw materials/ingredients.

These currently used analytical methods are state-of-the-art and represent the latest developments in technology. However, this work demonstrates that continuous method improvements for the nutrients identified must be pursued. In addition, this work supports a risk management approach that takes into consideration analytical method capability when establishing regulatory limits for nutrients in infant formulas. Ongoing efforts towards harmonization of regulatory requirements across global markets will facilitate evaluation of regulatory compliance in infant formulas.

1. Introduction

Infant formulas are the most strictly regulated food products and are rigorously tested for compliance with the applicable regulations. Nutrient composition in these products is defined to meet criteria for safety and suitability, minimizing risk of the deficit or excess. This is critically important considering the sensitive population these products serve.

The Codex Alimentarius (CODEX) Standard for Infant Formula and Formulas for Special Medical Purposes intended for infants (CXS 72-1981) together with the CODEX Standard for Follow-Up Formula (CXS 156-1987) contain provisions for essential composition, quality and safety factors for those products and constitute the international reference, which is the basis for many countries' national or regional regulations. In other cases, regulations developed by leading authorities such as the European Commission (e.g. EU Regulation 2006/141/EC; Bondoc, 2016a,b,c,d) are used in other parts of the world as a basis for their domestic regulation. Typically, there is no tolerance granted for values outside the regulatory limits. However, in addition to regulatory minimum (Min) and/or maximum (Max) levels for prescribed nutrients in these products, in many cases the national regulations include provisions for allowable tolerances relative to the label values for declared nutrients (LD). For example, tolerance on LD for added nutrients may be ± 20% in particular country regulations, e.g. Russia-Kazakhstan-Belarus (Customs Union, 2013), and Israel (Public Health Regulations, 2017, p. 5778). LD tolerances are not harmonized across majority of global regulatory bodies.

Infant formula producers must follow good manufacturing practices with strict process controls to ensure products are adequate, safe, and fully comply with regulatory requirements. As such, producers must consider several important aspects when establishing product specifications, which are defined as the Min and Max limits of a nutrient's content. For example, regulatory limits based on safety and nutritional considerations as well as quality and functional properties of the product must be taken into account. Also, regulatory requirements must be specifically met for each country in which the product is marketed. This can be quite challenging when a single product (with unique stock keeping unit) is sold in multiple countries with diverging regulations. In this case, producers must comply with all individual regulations by considering Min and Max limits as well as LD tolerances for each country. As a result, estimated product specifications may differ from country to country (Fig. 1). Altogether, these non-harmonized aspects of regulations across global markets represent a challenging environment for manufacturing in these product categories.

Compliance with regulations is generally accepted when the measured value of a particular nutrient is determined within the product specification for the relevant label declaration. In production, the regulatory compliance of a product can be assessed using the Process Capability Index (C_p) (Pyzdek & Keller, 2010, pp. 140–142), which expresses the extent to which the overall process variability fits within a product specification.

The overall variability observed in a finished product at time of manufacturing is comprised of three main sources of variability: from the materials/ingredients, manufacturing process and analytical method performance. Each of these sources must be strictly controlled to ensure product compliance.

The potential impact of analytical method variability on the assessment of product compliance is typically not considered when countries adopt new regulations or change existing ones. In several cases it is unclear whether state-of-the-art analytical methodologies and

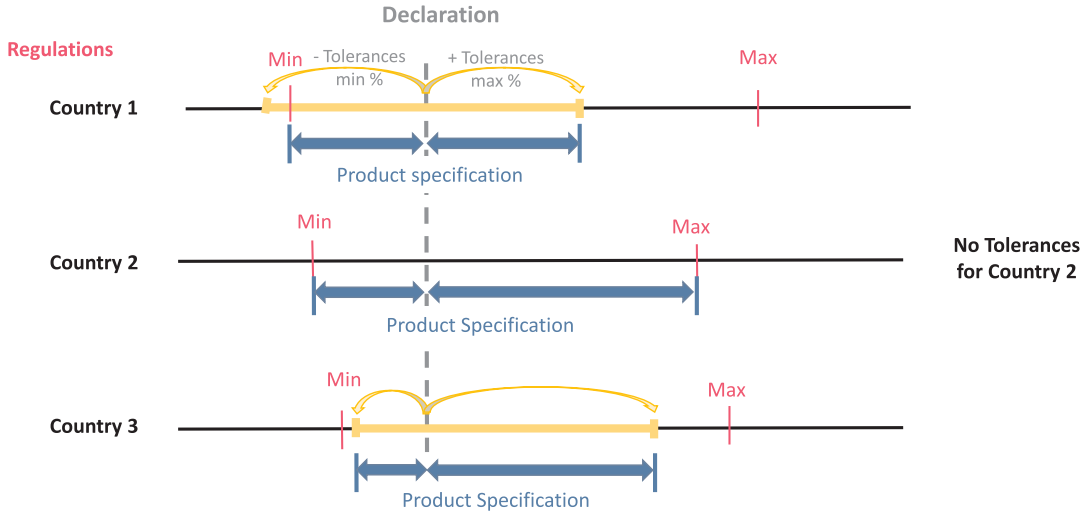

Fig. 1. Example of estimated product specification range for a given nutrient according to regulatory requirements in three countries with the same label declaration but different tolerances and Min and Max limits. Country 1: two-sided symmetrical tolerances around the declaration. Country 2: no tolerances around the declaration. Country 3: asymmetrical tolerances around the declaration.

their associated measurement uncertainties can support determination of regulatory compliance.

Recently, new standards for analytical methods on nutrients in infant formula (IF), Follow-Up Formula (FUF) and Formulas for Special Medical Purposes intended for infants (FSMP) have been jointly developed and published by AOAC INTERNATIONAL (AOAC), the International Organization for Standardization (ISO) and the International Dairy Federation (IDF) through the AOAC Stakeholder Panel on Infant Formula and Adult Nutritionals (SPIFAN) (Gill et al., 2015; Sullivan, 2012, 2016). As many as 15 of these new methods have been adopted or are in the process of being adopted as CODEX Type II methods, enabling them to be used for dispute resolution internationally. These methods are considered best in class for measuring nutrients in IF and adult nutrition with superior precision and trueness to any methods to date.

The objective of this study is to review global regulations for infant formulas and estimate if the determination of compliance with current regulatory requirements related to the Min and Max nutrient limits, together with LD tolerances, can be supported with SPIFAN methods and their inherent variability for nutrients in infant formulas. The focus will be on the following product categories: IF, FSMP intended for infants, and FUF including two subcategories as defined in CODEX (CXS 156-1987): FUF intended for older infants and young children (6–36 months) (FUF 6–36), and FUF for young children (1-3y) (FUF young children). The nutrients in focus are iron, copper, zinc, selenium, chromium, molybdenum, manganese, iodine, vitamins A, D, E, K_1, B_1, B_2, B_6, B_{12}, C and biotin, pantothenic acid, folic acid and inositol.

2. Materials & methods

Four steps were taken to evaluate the impact of analytical method variability on the assessment of product compliance with respect to global regulations:

1. Determination of theoretical product specifications based on the publicly available national regulations.
2. Review of analytical method variability of SPIFAN methods.
3. Definition of Method Capability (C_m).
4. Evaluation of C_m and conclusion on the ability of analytical methods to support determination of compliance with regulatory requirements for each nutrient.

2.1. Determination of theoretical product specifications

National regulations were reviewed for countries, products and nutrients as listed in Table 1. The chosen countries represent regions with the highest volumes of global IF sales (Euromonitor International, 2018). Regulations for FSMP were assessed in December 2016, while those for IF, FUF (6-36), and FUF young children were assessed in December 2017. For each country/regional regulations or guidelines, the Min and Max levels and tolerance(s) around the label declaration were assembled for each product category and nutrient when regulatory information existed. It should be noted that the EU has no harmonized tolerances for the product categories mentioned. Some countries like Germany, France, Italy, Denmark and Switzerland have their own specific tolerances, while others do not. Moreover, existing tolerances differ from one country to another and may have the status of guidance rather than regulation. Therefore, for the purpose of this paper the tolerance values as included in the European Commission, 2012(EC) guidance document (EC Guidance document, December 2012) were used. Although this guidance document excludes foods for particular nutritional uses as defined under Directive 2009/39/EC (incl. IF and FSMPs), the tolerance values for vitamins and minerals provide a good average basis for the exercise in this paper and are close to the tolerances employed by the European countries mentioned above.

Without true declared values, two-sided limits for regulation and/or tolerances were necessary to calculate product specification ranges for this theoretical exercise. Additionally, to have a fair assessment with respect to this study, the largest possible specification limits were defined by taking the midpoint of the regulatory requirements as the true value in the product and assuming the highest possible declared value by setting USL = Max regulatory limits. The specification range is expressed in percentage of the true value in the product since method variability and tolerances from declared values are often expressed as percentages.

A specification range was calculated for each country regulation and nutrient combination.

Depending on the available regulatory information, upper specification limit (*USL*), lower specification limit (*LSL*) and specification range can be ascertained according to Table 2.

The formula for LSL when both sides for regulatory and tolerance are available (Table 2) was established as follows and shown in Fig. 2. For the purpose of this paper, the widest interval for tolerance limits was estimated given the regulatory information.

By definition, if we note *Decl* the declared value, the tolerance interval lies between

$$Decl \times (1 - Tol_{min}) \tag{1}$$

and

$$Decl \times (1 + Tol_{max}) \tag{2}$$

and its size is:

$$Decl \times (Tol_{max} + Tol_{min}) \tag{3}$$

The following can be stated according to equation (3): the higher the *Decl*, the wider the interval of tolerance limits. When both sides for regulatory and tolerance are available, the *USL* cannot be higher than the regulatory maximum. Thus, to achieve the highest possible declared value we need to set USL as:

$$USL = Reg_{max} = Decl \times (1 + Tol_{max}) \tag{4}$$

From equation (4), *Decl* can be formulated as:

$$Decl = \frac{Reg_{max}}{1 + Tol_{max}} \tag{5}$$

Based on what was defined in (1), we can deduct from (5) in this case

Table 1
Countries, products and nutrients for which regulatory Min and Max levels and LD tolerances were collected.

Countries/constitutions	Products	Nutrients
Brazil	Infant formula (IF)	Vitamin A
China	Follow-up formula (FUF 6–36 months)	Vitamin B_1
Europe	Follow-Up Formula (FUF for young children) (1-3y)	Vitamin B_2
India	Foods for Special Medical Purposes (FSMP) intended for infants	Vitamin B_6
Indonesia		Vitamin B_{12}
Malaysia		Vitamin C
Mexico		Vitamin D
Pakistan		Vitamin E
Philippines		Vitamin K_1
Russia, Belarus and Kazakhstan		Folic acid
Singapore		Pantothenic acid
South Africa		Biotin
Thailand		Inositol
United States		Chromium
		Copper
		Iodine
		Iron
		Manganese
		Molybdenum
		Selenium
		Zinc

Table 2
Determination of upper specification limit (*USL*), lower specification limit (*LSL*) and specification range depending on available regulatory requirements.

Available regulatory information	USL	LSL	Specification range (%)
Both sides for regulation only	Reg_{Max}	Reg_{Min}	$\frac{(USL-LSL)}{(USL+LSL)} \times 200$
Both sides for tolerances only	$1 + Tol_{Max}$	$1 - Tol_{Min}$	*(USL-LSL)*
Both sides for regulation and tolerance[a]	Reg_{Max}	$Max\left(Reg_{Min}, Reg_{Max} \times \frac{1-Tol_{Min}}{1+Tol_{Max}}\right)$	$\frac{(USL-LSL)}{(USL+LSL)} \times 200$

Reg_{Min} = Lower regulatory limit in the unit of the analyte.
Reg_{Max} = Higher regulatory limit in the unit of the analyte.
Tol_{Min} = tolerance extent on the left side of the declared value expressed in percentage, e.g. +20%.
Tol_{Max} = tolerance extent on the right side of the declared value expressed in percentage, e.g. +30%.
USL = Upper Specification Limit.
LSL = Lower Specification Limit.
200 = is factor to express specification range as percentage by dividing by midpoint between USL and LSL, times 100.
[a] Tolerances are not allowed to go beyond regulatory limits.

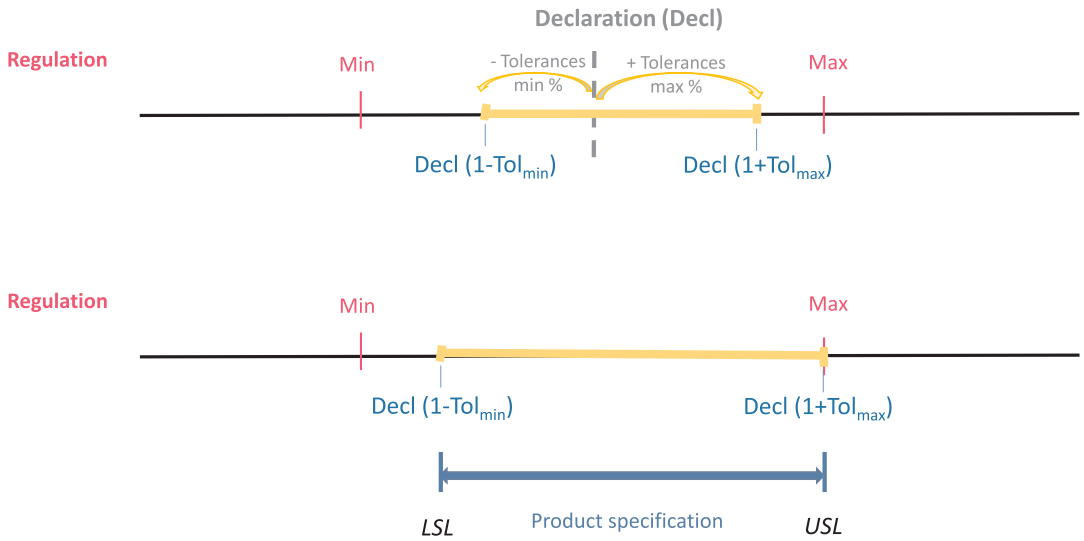

Fig. 2. Definition of largest possible specification limits based on regulations and tolerances.

Table 3
Analytical method variability (RSD_R) of SPIFAN methods for nutrients in infant formulas for given TTR.

Nutrient	Methods	Unit	Theoretical Target Range/100 kcal	Method Type	Mean RSD_R (%)	RSD_R SMPR® (%)
Vitamin A	AOAC 2012.10/ISO 20633	µg RE[a]	85–140	HPLC-UV	13.6	16
Vitamin B_1	AOAC 2015.14/ISO 21470	µg	70–110	HPLC-MS	10.1	10
Vitamin B_2	AOAC 2015.14/ISO 21470	mg	0.20–0.35	HPLC-MS/MS	6.7	10
Vitamin B_6	AOAC 2015.14/ISO 24470	µg	65–105	HPLC-MS/MS	9.3	10
Vitamin B_{12}	AOAC 2011.10/ISO 20634	µg	0.2–0.3	HPLC-UV	15.2	11
Vitamin C	AOAC 2012.22/ISO 20635	mg	12–20	(U)HPLC-UV	9.0	10
Vitamin D	AOAC 2016.05/ISO 20636	µg	1.7–2.8	HPLC-MS/MS	8.4	15
Vitamin E	AOAC 2012.10/ISO 20633	mg TE[b]	1.3–2.1	HPLC-FLD	7.8	16
Vitamin K_1	AOAC 2015.09/ISO 21446	µg	12.5–20.5	HPLC-FLD	7.9	10
Biotin	AOAC 2016.02/ISO 23305	µg	4.5–7.5	HPLC-UV	8.1	12
Folic acid	AOAC 2011.06	µg	20–32	HPLC-MS/MS	13.1	32
Inositol	AOAC 2011.18/ISO 20637	mg	6–10	HPLC-PAD	6.4	8
Pantothenic acid	AOAC 2012.16/ISO 20639	µg	560–940	UHPLC-MS/MS	4.8	15
Chromium	AOAC 2015.06/ISO 21424 | IDF 243)	µg	3.75–6.25	ICP-MS	4.9	15
Copper	AOAC 2015.06/ISO 21424 | IDF 243)	µg	65–105	ICP-MS	5.8	10
Iodine	AOAC 2012.15/ISO 20647 | IDF 234	µg	20–30	ICP-MS	9.0	15
Iron	AOAC 2015.06/ISO 21424 | IDF 243)	mg	0.85–1.45	ICP-MS	4.7	10
Manganese	AOAC 2015.06/ISO 21424 | IDF 243)	µg	34–43	ICP-MS	3.0	10
Molybdenum	AOAC 2015.06/ISO 21424 | IDF 243)	µg	3.75–6.25	ICP-MS	3.4	15
Selenium	AOAC 2015.06/ISO 21424 | IDF 243)	µg	4–6	ICP-MS	5.2	15
Zinc	AOAC 2015.06/ISO 21424 | IDF 243)	mg	0.75–1.25	ICP-MS	4.3	10

[a] RE = Retinol Equivalents.
[b] TE = Tocopherol Equivalents.

$$Lowest\ tolerance\ limit = Reg_{max} \times \frac{1 - Tol_{min}}{1 + Tol_{max}} \quad (6)$$

However, it should be avoided that the lowest tolerance limit is beyond Reg_{min}, and consequently, LSL is determined as

$$LSL = Max\left(Reg_{min}\ , Reg_{max} \times \frac{1 - Tol_{min}}{1 + Tol_{max}}\right) \quad (7)$$

2.2. Review of analytical method variability of SPIFAN methods

Analytical variability, also known as method precision, includes different variabilities such as repeatability (r) and reproducibility (R) (ISO 5725-1, 1994). The variability of an analytical method can be expressed in terms of standard deviation (SD) of repeatability (SD_r) and reproducibility (SD_R), relative standard deviation (RSD) of repeatability (RSD_r) and reproducibility (RSD_R) or limit (r or R).

Repeatability of an analytical method expresses the closeness of the results for a sample measured under identical conditions at the same time by the same analyst.

Reproducibility of an analytical method expresses the closeness of the results over the broadest possible range of conditions, under which the same samples are tested by the same method, but at different times, by different analysts in different laboratories. By definition $r \leq R$, and hence $RSD_r \leq RSD_R$.

To represent method variability independently from the laboratory using it, RSD_R values were used as estimates.

RSD_R values of SPIFAN methods were collected from the publication of multi-laboratory trial (MLT) data that are required for each SPIFAN method. The matrices studied for each MLT were a broad range of products (a minimum of 10) including milk-based IF, soy-based IF, hydrolyzed IF, FUF and FSMPs. To be more representative for the product category instead of using variability of a particular product, for which the method could have a particular low or high performance, the average of the analytical variabilities measured for different products was calculated. The value obtained from an MLT that includes many matrices with a relatively low number of laboratories is an estimation of the method SD for the defined product category. Analytical method variability is concentration dependent and therefore, the midpoint of regulatory requirements ± 25% was taken arbitrarily as a theoretical target range (TTR) for the calculation of the mean RSD_R. The mean RSD_R for each nutrient was calculated from the individual matrices validated including levels only within the TTR with the following formula:

$$Mean\ RSD_R = \frac{\sqrt{\frac{\sum_{i=1}^{n} df_i \times SD_{Ri}^2}{\sum_{i=1}^{n} df_i}}}{\sum_{i=1}^{n} df_i \times M_i} \quad (8)$$

where n is the number of matrices that the method was validated for at levels within the TTR. df_i is the number of degrees of freedom associated with the calculation of SD_{Ri}, M_i is the average level measured for the matrix i.

Table 3 summarizes mean RSD_R values. Although multiple official methods/standards are mentioned for a particular nutrient, they are technically equivalent, based on the same validation data and published by the respective standard developing organizations AOAC, ISO, and IDF in their own format. Before new official methods/standards are designated, the AOAC Stakeholder Panel aligns on performance requirements for these methods (AOAC INTERNATIONAL, 2019). Among others, the required limit of quantification (LOQ) and RSD_R, considering an analytical range based on regulatory requirements and current technologies available, are published in Standard Method Performance Requirements (SMPR®) for each nutrient. The required RSD_R values, stated in the corresponding SMPR®, at the given TTR are included in Table 3.

2.3. Definition of method capability (C_m)

Six Sigma is an initiative for continuous improvement developed by Motorola engineer, Bill Smith, in the mid-1980s (Snee, 2010). In this approach, sigma (σ), used by statisticians to denote SD, can be considered as process variability (Pyzdek & Keller, 2010, pp. 140–142), a concept representing the amount of variability relative to requirements or specifications. From this concept, the process capability index (C_p) can be defined as a measure of the ability of a process to produce results that meet specifications (Burnett et al., 1996). It represents the ratio between the acceptable spread (specifications) of results and the actual spread (process variability).

The process capability index compares the specifications for a given parameter to six times the estimated process variability SD_p.

$$Process\ capability\ C_p = \frac{Product\ specification\ range}{6 \times SD_p} \quad (9)$$

Assuming the results are normally distributed and the product is manufactured on target, it is possible to derive a direct link between the C_p and expected percentage of products out of specification. For instance, if a parameter (nutrient) in a production process has a C_p of 1, it is expected that 0.27% of the products will be out of specifications for the parameter (nutrient) of interest based on 99% confidence level.

Applying the same principle, the impact of analytical method variability on the assessment of product compliance can be assessed for a given analytical method by defining a Method Capability index (C_m), determining to which extent a method can demonstrate compliance (Bais, 2008; Dejaegher et al., 2006).

The method capability can be defined as:

$$Method\ capability\ C_m = \frac{Product\ specification\ range}{6 \times SD_m} \quad (10)$$

where SD_m is the method standard deviation.

This can be equivalently expressed as:

$$C_m = \frac{Product\ specification\ range\ (\%)}{6 \times RSD_{(R)}\ (\%)} \quad (11)$$

Assuming the normal distribution, a direct link can be derived between the C_m value and expected percentage of values out of specifications due to method variability in much the same ways as C_p.

The relationship between specification range, process variability and analytical variability is illustrated in Fig. 3.

Since the method variability is included in the overall process variability $SD_m < SD_p$, and consequently, $C_m > C_p$.

The requirement for C_m to achieve the required C_p directly depends on the weight of the method variance within the overall variability as:

$$C_m = C_p \times \sqrt{\frac{1}{w}} \quad (12)$$

where w represents the proportion of total variance attributable to measurement variance.

The variance of an analytical method is chosen arbitrarily to be $w = 1/3$ of the overall process variance, where the other 2/3 are considered to come from ingredient and manufacturing process variability, respectively. Consequently, a C_m should be at least $1.73C_p$. In that case, a $C_m < 1$ would be critical. By considering a weight of the method variance equal to 1/3, it means that any method with $C_m < 1.73$ would become a problem for achieving a C_p of 1 (0.27% products out of specification range) and a $C_m < 1$ would be critical. It should be noted that the 1/3 factor can vary depending on analytical method/analyte, matrix and production process.

3. Results

Tables 4–7 include the individual results of the specification range

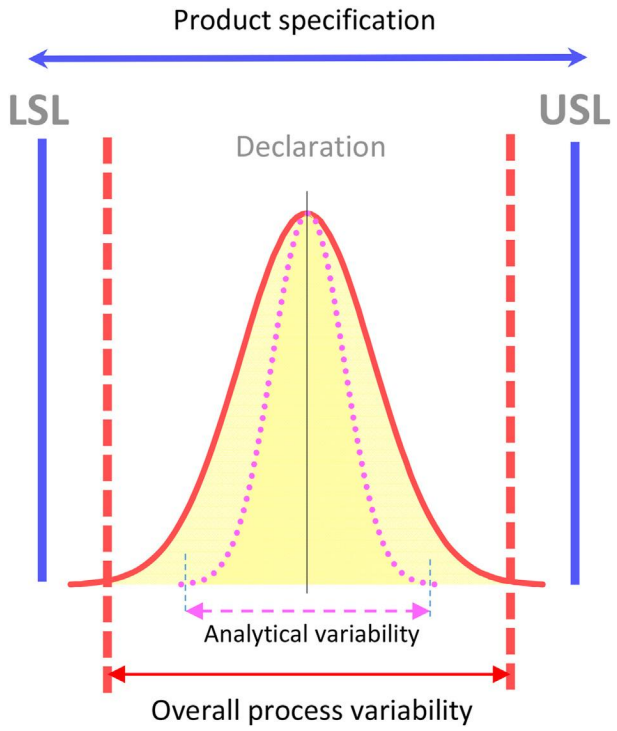

Fig. 3. Illustration expressing the relationship between product specification, process variability and analytical variability.

and C_m for each product/country-region/nutrient combination and highlights specific concerns. Table 8 groups all the individual results to indicate, for each nutrient, the frequency of individual country/region regulations for which $C_m < 1$, $1 \leq C_m < 1.73$ and $C_m \geq 1.73$. These results show that for IF, 54 (27%) nutrient/country or region regulatory requirements combinations are at risk of not reaching a C_p of 1 since $C_m < 1.73$. In these cases, there is a high risk of obtaining analytical results outside of specification ranges, even though the product is truly compliant with product specifications. For FUF 6–36, FUF young children and FSMP, the number of nutrient/country or region regulatory requirements combinations with $C_m < 1.73$ are 38 (35%), 40 (41%) and 55 (28%), respectively.

The results in Table 8 also show that vitamins A, B_{12}, D and folic acid in product categories IF, FUF 6–36, FUF young children and FSMP have the highest number of country/region regulations with $C_m < 1.73$, raising concerns upon meeting the requirements using the SPIFAN methods. Several country/region regulations for these nutrients show a $C_m < 1$, which can be considered critical, including China, some EU member states, Pakistan, Russia, Singapore, South Africa and Thailand.

In addition, for the categories FUF young children and/or FSMP, three or more country regulations have $C_m < 1.73$ for vitamin B_1, B_6, C and biotin. Furthermore, vitamin B_1 and B_6 in FSMP and Russian regulation have $C_m < 1$.

Figs. 4–7 show product specification ranges (LSL, USL) for IF to help visualize the diversity in country/region regulatory requirements. Vitamins A, B_{12}, D and folic acid are used as examples because these nutrients have the highest number of countries/regions with $C_m < 1.73$. The figures confirm observations that country/region regulatory requirements for some EU member states, Pakistan, Russia, Singapore, South Africa and Thailand are generally narrower compared to other country/regional regulatory requirements for one or more nutrients mentioned.[1]

[1] [1] RE = Retionol Equivalents

For the product/nutrient/country combinations with $C_m < 1$, the probability of obtaining an analytical result out of the specification range due to analytical variability was calculated. Assumptions were that only one, single analysis is done in one finished product with a true nutrient value in the middle of the specification range. Table 9 summarizes the percentage of products out of specification limits LSL/USL due only to analytical method variability in the cases where $C_m < 1$. In summary, the probability of obtaining an analytical result out of the specification range due to analytical variability was the highest for vitamin A with European legislation for IF (8%), vitamin A with European legislation for FUF (6-36) (8%), vitamin A with China regulation for FUF young children (1.4%) and vitamin A with Russian regulation for FSMP (8%).

4. Discussion

To comply with national regulations and ensure products are manufactured within product specifications, accurate analytical testing is required. Most legacy test methods for IF originated from AOAC and were developed and validated in the 1980s after the Infant Formula Act of 1980 was enacted in the United States. Although these methods performed well for many years, IF compositions changed over time and new products may now contain optional ingredients that challenge the performance of legacy test methods. As a result, there were several anecdotal cases (not published) recently in which by using the legacy AOAC methods led to disputes with regulatory agencies because the methods were no longer fit for purpose for some IF. Moreover, instrument technology has advanced dramatically over the past four decades and new analytical methodologies exhibiting better selectivity, specificity and sensitivity have been developed, providing better method performance versus legacy methods. In 2010, AOAC reached an agreement with the Infant Nutrition Council of America (formerly International Formula Council) to establish the SPIFAN and a goal to modernize AOAC IF test methods. The agreement led to the development of Standard Method Performance Requirements (SMPR®) for 30 nutrients. Based on the approved SMPRs®, methods were solicited, evaluated, validated, and approved through the AOAC *Official Methods* ℠ process (Sullivan, 2016). To date, 15 SPIFAN Official Method Final Action methods and their comparable ISO/IDF standards covering 21 nutrients have been adopted as Type II reference methods by the Codex Alimentarius Commission (CXS 234, 1999). These methods are considered state of the art because of the rigorous process by which they were selected, involving a wide range of internationally relevant stakeholders. Three important parts of an SMPR® are the applicability statement, the analytical technique required and method performance requirements. The applicability of an Official Method considers the analytes which, according to regulatory international references/national regulations, must be quantified. However, agreeing on the applicability statement can be challenging as sometimes international regulatory documents do not adequately define target nutrient forms (e.g. folate, inositol, vitamin E in CXS 72–1981). The analytical technique considers present technology that can best quantify analytes mentioned in the applicability statement. A prerequisite for becoming an Official Method is to meet established performance requirements. To validate the methods, a suite of 12 test materials were developed to represent the majority of matrices on the market. These included IF, FUF, FSMP and a National Institute of Science and Technology Standard Reference Material. These materials were used in single laboratory validation (SLV) and MLT studies. From Table 3 it can be concluded that almost all Official Methods meet the SMPR® requirements. However, one exception is the method for vitamin B_{12}, which has an RSD_R of 15% that is above the SMPR® target of 11%. The AOAC Expert Review Panel for SPIFAN nutrient methods concluded that for the time being, and before any new developments are made on the analysis of vitamin B_{12} in such products, the method demonstrated acceptable repeatability and reproducibility (Butler-Thompson et al., 2015).

Table 4
Results of method capability (C_m) on infant formula for each country/nutrient combination calculated using relative standard deviation (RSD_R) of the recommended method (Table 3). Where no two-sided limits or tolerances were available for a particular country/region, C_m could not be set (noted as "ok"). Highlighted as critical (red) are $C_m < 1$, while highlighted as concern (orange) are $1 < C_m < 1.73$.

	Brazil	China	Europe	India	Indonesia	Malaysia	Mexico	Pakistan	Philippines	Russia, Belarus, Kazakhstan	Singapore	South Africa	Thailand	United States
vitamin A														
Spec Range (%)	100	101	48		100	100	100	77	100	86	67	75	67	100
C_m	1.22	1.24	0.59	ok	1.22	1.22	1.22	0.94	1.22	1.05	0.82	0.92	0.82	1.22
vitamin B_1														
Spec Range (%)	133	134	79		133	133	133	133	133	136		75		
C_m	2.19	2.21	1.30	ok	2.19	2.19	2.19	2.19	2.19	2.24	ok	1.24	ok	ok
vitamin B_2														
Spec Range (%)	145	145	79		145	145	145	145	145	139		75		
C_m	3.58	3.58	1.95	ok	3.58	3.58	3.58	3.58	3.58	3.43	ok	1.85	ok	ok
vitamin B_6														
Spec Range (%)	133	136	79		133	133	133	133	133	107		75		
C_m	2.37	2.43	1.41	ok	2.37	2.37	2.37	2.37	2.37	1.91	ok	1.34	ok	ok
vitamin B_{12}														
Spec Range (%)	175	173	79		175	175	175	175	175	100		75		
C_m	1.91	1.89	0.86	ok	1.91	1.91	1.91	1.91	1.91	1.09	ok	0.82	ok	ok
vitamin C														
Spec Range (%)	100	149	79		150	150	150	150	150	93		61		
C_m	1.84	2.74	1.46	ok	2.76	2.76	2.76	2.76	2.76	1.71	ok	1.12	ok	ok
vitamin D														
Spec Range (%)	86	82	40		86	86	86	86	86	50	86	75	67	86
C_m	1.71	1.63	0.79	ok	1.71	1.71	1.71	1.71	1.71	0.99	1.71	1.49	1.33	1.71
vitamin E														
Spec Range (%)	164	164	79		164	164	164	164	164	100		75		
C_m	3.52	3.52	1.69	ok	3.52	3.52	3.52	3.52	3.52	2.14	ok	1.61	ok	ok
vitamin K_1														
Spec Range (%)	148	146	79		148	148	148	148	148	120		75		
C_m	3.13	3.09	1.67	ok	3.13	3.13	3.13	3.13	3.13	2.54	ok	1.59	ok	ok
Folic acid														
Spec Range (%)	133	131	79		133	133	133	117	133	141		75		
C_m	1.70	1.67	1.01	ok	1.70	1.70	1.70	1.49	1.70	1.80	ok	0.96	ok	ok
Pantothenic acid														
Spec Range (%)	133	133	79		133	133	133	133	133	135		75		
C_m	4.62	4.62	2.75	ok	4.62	4.62	4.62	4.62	4.62	4.69	ok	2.61	ok	ok
Biotin														
Spec Range (%)	148	148	79		148	148		148	148	120		75		
C_m	3.04	3.04	1.62	ok	3.04	3.04	ok	3.04	3.04	2.47	ok	1.54	ok	ok
Inositol														
Spec Range (%)	164	162	79		164	164	164	164		173		75		
C_m	4.27	4.22	2.06	ok	4.27	4.27	4.27	4.27	ok	4.51	ok	1.95	ok	ok
Copper														
Spec Range (%)	110	109	50		110	110	110	108	145	67		61		
C_m	3.19	3.16	1.45	ok	3.19	3.19	3.19	3.13	4.20	1.94	ok	1.77	ok	ok
Chromium														
Spec Range (%)														
C_m	ok	ok	ok	ok	ok	ok	ok	ok	ok	ok	ok	ok	ok	ok
iodine														
Spec Range (%)	143	139	64		143	143	143	100		100		61	175	175
C_m	2.66	2.59	1.19	ok	2.66	2.66	2.66	1.86	ok	1.86	ok	1.14	3.26	3.26
Iron														
Spec Range (%)	97	113	76		127			108		100		61	181	181
C_m	3.41	3.97	2.67	ok	4.46	ok	ok	3.79	ok	3.51	ok	2.14	6.35	6.35
Manganese														
Spec Range (%)	196	181	76		196	196	196	196	196	187		61		
C_m	10.99	10.15	4.08	ok	10.99	10.99	10.99	10.99	10.99	10.48	ok	3.42	ok	ok
Molybdenum														
Spec Range (%)			76											
C_m	ok	ok	4.10	ok	ok	ok	ok	ok	ok	ok	ok	ok	ok	ok
Selenium														
Spec Range (%)	160	119	76		160	160	160	100	160	120	133	61		111
C_m	5.09	3.79	2.42	ok	5.09	5.09	5.09	3.18	5.09	3.82	4.23	1.94	ok	3.53
Zinc														
Spec Range (%)	100	100	67		100	100	100	100	100	108		61		
C_m	3.90	3.90	2.61	ok	3.90	3.90	3.90	3.90	3.90	4.21	ok	2.38	ok	ok

Table 5
Results of method capability (C_m) on follow-up formula (6-36) for each country/nutrient combination calculated using relative standard deviation (RSD_R) of the recommended method (Table 3). Where no two-sided limits or tolerances were available for a particular country/region, C_m could not be set (noted as "ok"). Highlighted as critical (red) are $C_m < 1$, while highlighted as concern (orange) are $1 < C_m < 1.73$.

	Brazil	China	Europe	India	Indonesia	Malaysia	Mexico	Pakistan	Philippines	Russia, Belarus, Kazakhstan	Singapore	South Africa	Thailand	United States
vitamin A														
Spec Range (%)	100		48			100	100	95		86	67	75	100	100
C_m	1.22	ok	0.59	ok	ok	1.22	1.22	1.16	ok	1.05	0.82	0.92	1.22	1.22
vitamin B_1														
Spec Range (%)			79					133		136		75		
C_m	ok	ok	1.50	ok	ok	ok	ok	2.19	ok	2.24	ok	1.24	ok	ok
vitamin B_2														
Spec Range (%)	145		79					145		135		75		
C_m	3.58	ok	1.95	ok	ok	ok	ok	3.58	ok	3.33	ok	1.85	ok	ok
vitamin B_6														
Spec Range (%)	100		79					127		100		75		
C_m	1.78	ok	1.41	ok	ok	ok	ok	2.27	ok	1.78	ok	1.34	ok	ok
vitamin B_{12}														
Spec Range (%)	133		79					172		100		75		
C_m	1.45	ok	0.86	ok	ok	ok	ok	1.88	ok	1.09	ok	0.82	ok	ok
vitamin C														
Spec Range (%)	100		79					127		93		61		
C_m	1.84	ok	1.46	ok	ok	ok	ok	2.34	ok	1.71	ok	1.12	ok	ok
vitamin D														
Spec Range (%)	100		40			100	100	111		73	86	75	100	86
C_m	1.99	ok	0.79	ok	ok	1.99	1.99	2.21	ok	1.45	1.71	1.49	1.99	1.71
vitamin E														
Spec Range (%)	164		79					164		133		75		
C_m	3.52	ok	1.69	ok	ok	ok	ok	3.52	ok	2.85	ok	1.61	ok	ok
vitamin K_1														
Spec Range (%)	148		79					148		149		75		
C_m	3.13	ok	1.67	ok	ok	ok	ok	3.13	ok	3.15	ok	1.59	ok	ok
Folic acid														
Spec Range (%)	133		79					117		141		75		
C_m	1.70	ok	1.01	ok	ok	ok	ok	1.49	ok	1.80	ok	0.96	ok	ok
Pantothenic acid														
Spec Range (%)	133		79					133		129		75		
C_m	4.62	ok	2.75	ok	ok	ok	ok	4.62	ok	4.48	ok	2.61	ok	ok
Biotin														
Spec Range (%)	148		79					148		120		75		
C_m	3.04	ok	1.52	ok	ok	ok	ok	3.04	ok	2.47	ok	1.54	ok	ok
Inositol														
Spec Range (%)			79					164		173		75		
C_m	ok	ok	2.06	ok	ok	ok	ok	4.27	ok	4.51	ok	1.95	ok	ok
Copper														
Spec Range (%)	110		76					80		91		61		
C_m	3.19	ok	2.20	ok	ok	ok	ok	2.32	ok	2.64	ok	1.77	ok	ok
Chromium														
Spec Range (%)														
C_m	ok	ok	ok	ok	ok	ok	ok	ok	ok	ok	ok	ok	ok	ok
iodine														
Spec Range (%)			64					100		150		61		175
C_m	ok	ok	1.19	ok	ok	ok	ok	1.86	ok	2.79	ok	1.14	ok	3.26
Iron														
Spec Range (%)	94		76			67	67	67		67		61	67	181
C_m	3.30	ok	2.67	ok	ok	2.35	2.35	2.35	ok	2.35	ok	2.14	2.35	6.35
Manganese														
Spec Range (%)			76					192		187		61		
C_m	ok	ok	4.26	ok	ok	ok	ok	10.67	ok	10.48	ok	3.42	ok	ok
Molybdenum														
Spec Range (%)			76											
C_m	ok	ok	3.71	ok	ok	ok	ok	ok	ok	ok	ok	ok	ok	ok
Selenium														
Spec Range (%)	160		76					100		120	133	61		
C_m	5.09	ok	2.42	ok	ok	ok	ok	3.18	ok	3.82	4.23	1.94	ok	ok
Zinc														
Spec Range (%)	100		50					100		85		61		
C_m	3.90	ok	1.95	ok	ok	ok	ok	3.90	ok	3.32	ok	2.38	ok	ok

The method performance requirements (analytical range, LOQ and precision parameters) also consider compositional and tolerance requirements. However, no procedure has been established to set performance requirements for a method to deliver results which fit with different global regulations. The concept of C_m described in this paper is an adequate predictive tool to assist in setting these requirements. A

Table 6
Results of method capability (C_m) on follow-up formula for young children for each country/nutrient combination calculated using relative standard deviation (RSD_R) of the recommended method (Table 3). Where no two-sided limits or tolerances were available for a particular country/region, C_m could not be set (noted as "ok"). Highlighted as critical (red) are $C_m < 1$, while highlighted as concern (orange) are $1 < C_m < 1.73$.

	Brazil	China	Europe	India	Indonesia	Malaysia	Mexico	Pakistan	Philippines	Russia, Belarus, Kazakhstan	Singapore	South Africa	Thailand	United States
vitamin A														
Spec Range (%)		67	79		100		100		100	107		75		
C_m	ok	0.82	0.97	ok	1.22	ok	1.22	ok	1.22	1.31	ok	0.92	ok	ok
vitamin B_1														
Spec Range (%)		67	79				100			107		75		
C_m	ok	1.11	1.30	ok	ok	ok	1.65	ok	ok	1.77	ok	1.24	ok	ok
vitamin B_2														
Spec Range (%)		55	79				100			107		75		
C_m	ok	1.36	1.95	ok	ok	ok	2.47	ok	ok	2.64	ok	1.85	ok	ok
vitamin B_6														
Spec Range (%)		67	79				100			107		75		
C_m	ok	1.20	1.41	ok	ok	ok	1.78	ok	ok	1.91	ok	1.34	ok	ok
vitamin B_{12}														
Spec Range (%)			79				100			107		75		
C_m	ok	ok	0.86	ok	ok	ok	1.09	ok	ok	1.17	ok	0.82	ok	ok
vitamin C														
Spec Range (%)		67	79				100			107		75		
C_m	ok	1.23	1.46	ok	ok	ok	1.84	ok	ok	1.97	ok	1.38	ok	ok
vitamin D														
Spec Range (%)		67	79		100		100		100	107		75		
C_m	ok	1.33	1.57	ok	1.99	ok	1.99	ok	1.99	2.13	ok	1.49	ok	ok
vitamin E														
Spec Range (%)		66	79				100			107		75		
C_m	ok	1.41	1.69	ok	ok	ok	2.14	ok	ok	2.29	ok	1.61	ok	ok
vitamin K_1														
Spec Range (%)			79				100			107		75		
C_m	ok	ok	1.67	ok	ok	ok	2.11	ok	ok	2.26	ok	1.59	ok	ok
Folic acid														
Spec Range (%)		67	79				100			107		75		
C_m	ok	0.85	1.01	ok	ok	ok	1.28	ok	ok	1.37	ok	0.96	ok	ok
Pantothenic acid														
Spec Range (%)		67	79				100			107		75		
C_m	ok	2.33	2.75	ok	ok	ok	3.48	ok	ok	3.72	ok	2.61	ok	ok
Biotin														
Spec Range (%)		67	79				100			107		75		
C_m	ok	1.38	1.62	ok	ok	ok	2.06	ok	ok	2.20	ok	1.54	ok	ok
Inositol														
Spec Range (%)			79				100			107		75		
C_m	ok	ok	2.06	ok	ok	ok	2.61	ok	ok	2.79	ok	1.95	ok	ok
Copper														
Spec Range (%)			79				100			107		75		
C_m	ok	ok	2.29	ok	ok	ok	2.90	ok	ok	3.10	ok	2.17	ok	ok
Chromium														
Spec Range (%)														
C_m	ok	ok	ok	ok	ok	ok	ok	ok	ok	ok	ok	ok	ok	ok
iodine														
Spec Range (%)			79				100			107		75		
C_m	ok	ok	1.47	ok	ok	ok	1.86	ok	ok	1.99	ok	1.40	ok	ok
Iron														
Spec Range (%)		67	79		67		100		67	107		67		
C_m	ok	2.35	2.77	ok	2.35	ok	3.51	ok	2.35	3.76	ok	2.35	ok	ok
Manganese														
Spec Range (%)			79				100			107		75		
C_m	ok	ok	4.43	ok	ok	ok	5.61	ok	ok	6.00	ok	4.20	ok	ok
Molybdenum														
Spec Range (%)			79				100			107		75		
C_m	ok	ok	3.86	ok	ok	ok	4.88	ok	ok	5.22	ok	3.66	ok	ok
Selenium														
Spec Range (%)			79				100			107		75		
C_m	ok	ok	2.52	ok	ok	ok	3.18	ok	ok	3.41	ok	2.39	ok	ok
Zinc														
Spec Range (%)		67	79				100			107		75		
C_m	ok	2.61	3.08	ok	ok	ok	3.90	ok	ok	4.17	ok	2.93	ok	ok

target precision for an analytical method can be designated based on a required C_m and regulatory requirements and tolerances from the label declaration, taking into consideration the overall variability of a product, which includes variability from the ingredient sources combined with production processes.

Considering the variability of nutrient levels attributed to

Table 7
Results of method capability (C_m) on foods for special medical purposes (FSMP) intended for infants for each country/nutrient combination calculated using relative standard deviation (RSD_R) of the recommended method (Table 3). Where no two-sided limits or tolerances were available for a particular country/region, C_m could not be set (noted as "ok"). Highlighted as critical (red) are $C_m < 1$, while highlighted as concern (orange) are $1 < C_m < 1.73$.

	Brazil	China	Europe	India	Indonesia	Malaysia	Mexico	Pakistan	Philippines	Russia, Belarus, Kazakhstan	Singapore	South Africa	Thailand	United States
vitamin A														
Spec Range (%)	100	101	79		100	100	100	100	100	48	67	75	75	100
C_m	1.22	1.24	0.97	ok	1.22	1.22	1.22	1.22	1.22	0.59	0.82	0.92	0.92	1.22
vitamin B_1														
Spec Range (%)	133	151	79		133	133	133	133	133	40		75		
C_m	2.19	2.49	1.30	ok	2.19	2.19	2.19	2.19	2.19	0.66	ok	1.24	ok	ok
vitamin B_2														
Spec Range (%)	145	158	108		145	145	145	145	145	50		75		
C_m	3.58	3.90	2.67	ok	3.58	3.58	3.58	3.58	3.58	1.23	ok	1.85	ok	ok
vitamin B_6														
Spec Range (%)	133	159	79		133	133	133	133	133	34		75		
C_m	2.37	2.84	1.41	ok	2.37	2.37	2.37	2.37	2.37	0.61	ok	1.34	ok	ok
vitamin B_{12}														
Spec Range (%)	175	174	79		175	175	175	175	175	67		75		
C_m	1.91	1.90	0.88	ok	1.91	1.91	1.91	1.91	1.91	0.73	ok	0.82	ok	ok
vitamin C														
Spec Range (%)	100	160	79		150	150	150	150	150	100		61		
C_m	1.84	2.95	1.46	ok	2.76	2.76	2.76	2.76	2.76	1.84	ok	1.12	ok	ok
vitamin D														
Spec Range (%)	86	100	40		86	86	86	86	86	78	86	75	67	86
C_m	1.71	1.99	0.79	ok	1.71	1.71	1.71	1.71	1.71	1.55	1.71	1.49	1.33	1.71
vitamin E														
Spec Range (%)	164	164	79		164	164	164	164	164	80		75		
C_m	3.52	3.52	1.69	ok	3.52	3.52	3.52	3.52	3.52	1.72	ok	1.61	ok	ok
vitamin K_1														
Spec Range (%)	148	146	79		148	148	148	148	148			75		
C_m	3.13	3.09	1.67	ok	3.13	3.13	3.13	3.13	3.13	ok	ok	1.59	ok	ok
Folic acid														
Spec Range (%)	133	169	79		133	133	133	133	133	67		75		
C_m	1.70	2.16	1.01	ok	1.70	1.70	1.70	1.70	1.70	0.85	ok	0.96	ok	ok
Pantothenic acid														
Spec Range (%)	133	158	79		133	133	133	133	133			75		
C_m	4.62	5.49	2.75	ok	4.62	4.62	4.62	4.62	4.62	ok	ok	2.61	ok	ok
Biotin														
Spec Range (%)	148	170	79		148	148	148	148	148			75		
C_m	3.04	3.50	1.62	ok	3.04	3.04	3.04	3.04	3.04	ok	ok	1.54	ok	ok
Inositol														
Spec Range (%)	164	162			164	164	164	164	164					
C_m	4.27	4.22	ok	ok	4.27	4.27	4.27	4.27	4.27	ok	ok	ok	ok	ok
Copper														
Spec Range (%)	110	143	76		110	110	110	110	110	108		61		
C_m	3.19	4.14	2.20	ok	3.19	3.19	3.19	3.19	3.19	3.13	ok	1.77	ok	ok
Chromium														
Spec Range (%)														
C_m	ok	ok	ok	ok	ok	ok	ok	ok	ok	ok	ok	ok	ok	ok
iodine														
Spec Range (%)	143	168	76		143	143	143	143	143			61	175	175
C_m	2.66	3.13	1.41	ok	2.66	2.66	2.66	2.66	2.66	ok	ok	1.14	3.26	3.26
Iron														
Spec Range (%)	97	133	76		127					80		61	181	181
C_m	3.41	4.67	2.67	ok	4.46	ok	ok	ok	ok	2.81	ok	2.14	6.35	6.35
Manganese														
Spec Range (%)	196	191	76		196	196	196	196	196			61	196	
C_m	10.99	10.71	4.26	ok	10.99	10.99	10.99	10.99	10.99	ok	ok	3.42	10.99	ok
Molybdenum														
Spec Range (%)			76			103						67		
C_m	ok	ok	3.71	ok	ok	5.03	ok	ok	ok	ok	ok	3.27	ok	ok
Selenium														
Spec Range (%)	160	153	76		160	160	160	160	160		158	61	160	111
C_m	5.09	4.87	2.42	ok	5.09	5.09	5.09	5.09	5.09	ok	5.03	1.94	5.09	3.53
Zinc														
Spec Range (%)	100	134	100		100	100	100	100	100	108		61		
C_m	3.90	5.23	3.90	ok	3.90	3.90	3.90	3.90	3.90	4.21	ok	2.38	ok	ok

Table 8
Frequency (in number of countries/regions) of country/region regulatory requirements with $C_m < 1$, $1 \leq C_m < 1.73$, $C_m \geq 1.73$ for product nutrient combinations. Highlighted as critical (red) are $C_m < 1$, while highlighted as concern (orange) are $1 < C_m < 1.73$.

	Infant formula			Follow-up formula (6-36)			Follow-up Formula young children			FSMP intended for infants		
	C_m <1	$1 \leq C_m < 1.73$	$C_m \geq 1.73$ or NA	C_m <1	$1 \leq C_m < 1.73$	$C_m \geq 1.73$ or NA	C_m <1	$1 \leq C_m < 1.73$	$C_m \geq 1.73$ or NA	C_m <1	$1 \leq C_m < 1.73$	$C_m \geq 1.73$ or NA
	N countries	N countries	N countries	N countries	N countries	N countries	N countries	N countries	N countries	N countries	N countries	N countries
vitamin A	5	8	0	3	7	0	3	4	0	5	8	0
vitamin B_1	0	2	8	0	2	2	0	4	1	1	2	7
vitamin B_2	0	0	10	0	0	5	0	1	4	0	1	9
vitamin B_6	0	2	8	0	2	3	0	3	2	1	2	7
vitamin B_{12}	2	1	7	2	2	1	2	2	0	3	0	7
vitamin C	0	3	7	0	3	2	0	3	2	0	2	8
vitamin D	2	11	0	1	4	5	0	3	4	1	11	1
vitamin E	0	2	8	0	2	3	0	3	2	0	3	7
vitamin K_1	0	2	8	0	2	3	0	2	2	0	2	7
Folic acid	1	8	1	1	3	1	2	3	0	2	7	1
Pantothenic acid	0	0	10	0	0	5	0	0	5	0	0	9
Biotin	0	2	7	0	2	3	0	3	2	0	2	7
Inositol	0	0	9	0	0	4	0	0	4	0	0	7
Copper	0	1	9	0	0	5	0	0	4	0	0	10
iodine	0	2	9	0	2	3	0	2	2	0	2	9
Iron	0	0	9	0	0	9	0	0	7	0	0	8
Manganese	0	0	10	0	0	4	0	0	4	0	0	10
Molybdenum	0	0	1	0	0	1	0	0	4	0	0	3
Selenium	0	0	12	0	0	6	0	0	4	0	0	12
Zinc	0	0	10	0	0	5	0	0	5	0	0	10
Total frequency	54		143	38		70	40		58	55		139

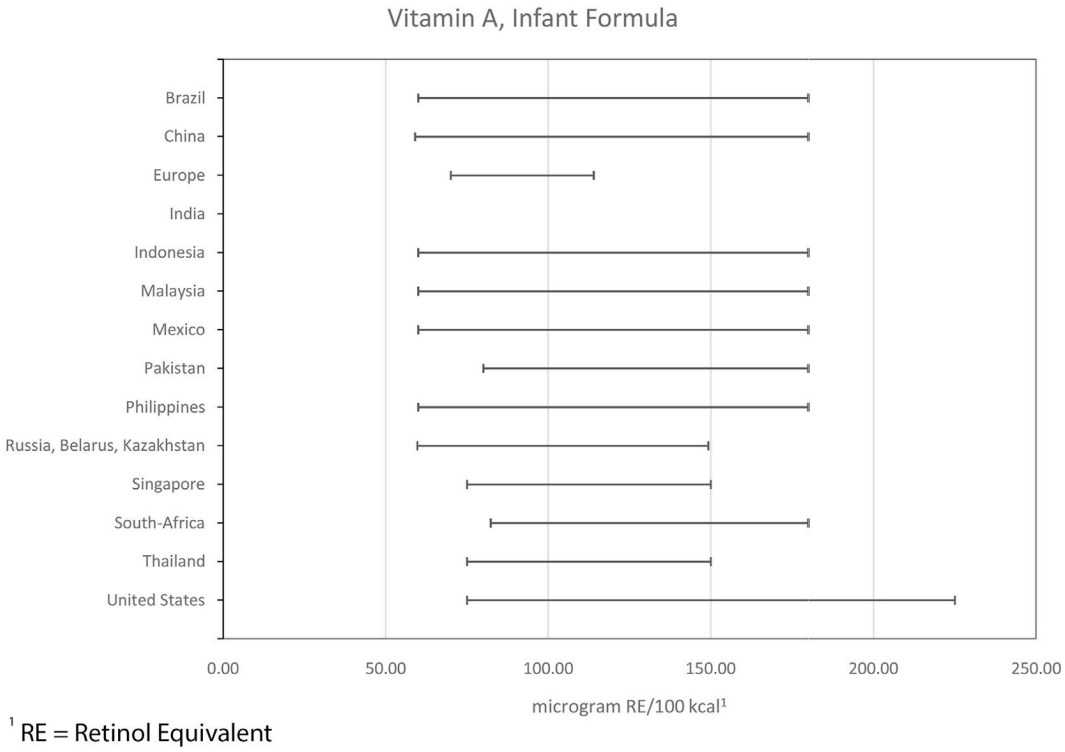

Fig. 4. Product specification range (LSL, USL) for vitamin A in IF and country/region regulatory requirements.

ingredients, vitamins and minerals used to fortify food products, these are generally introduced via a premix that contains multiple nutrients in a single commodity, with each nutrient present at a high concentration. The specification range of each nutrient in the premix is generally within ± 15% of the target for nutrients most vulnerable to decomposition (e.g. vitamin A), and typically ± 10% for other nutrients. Assuming a C_p of 1 for a single vitamin or mineral produced by the ingredient manufacturer, an ingredient variability $\leq$5% is to be expected. In addition, it is not uncommon for the variability of the production process to add a few percentage points to the overall product variability, especially when nutrients are being added by dry mixing. In addition to premixes, natural ingredients of biological origin inherently vary in composition.

Particle size distribution can be another source of variability when testing nutrients in dry blended mixtures such as IF. If small sample sizes (e.g. 0.5 g) are taken from powdered, dry-blended formulas for analysis, the risk of an analytical value out of the specification range is high due to a potential heterogeneous distribution of nutrients. To

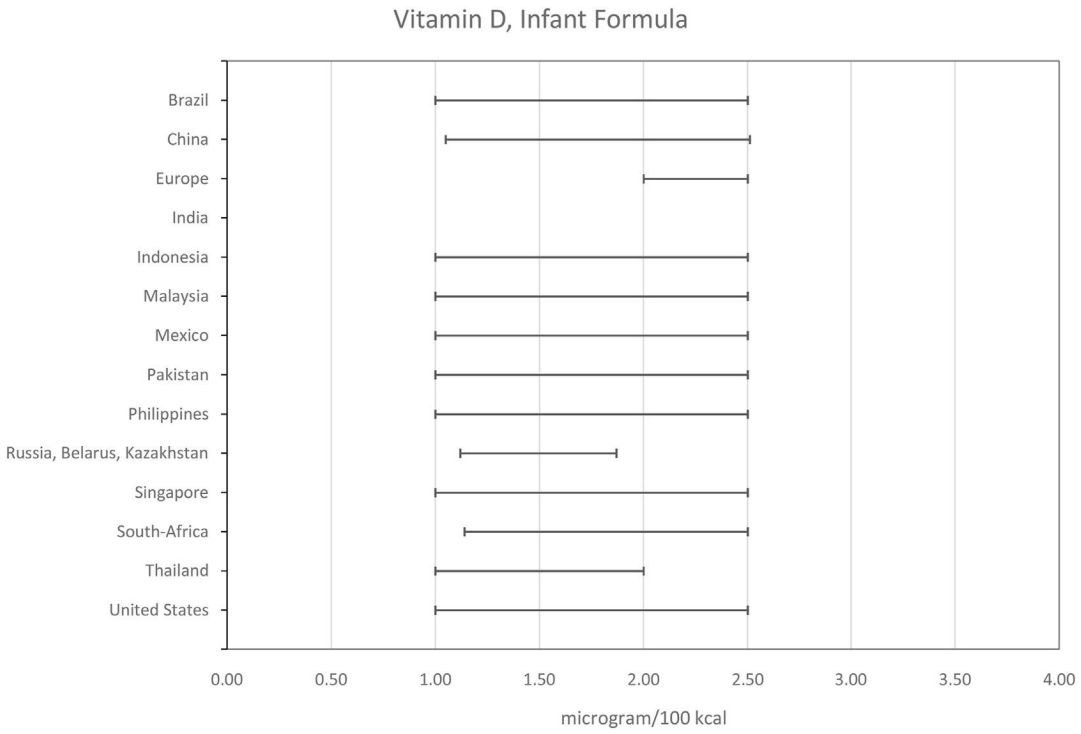

Fig. 5. Product specification range (LSL, USL) for vitamin D in IF and country/region regulatory requirements.

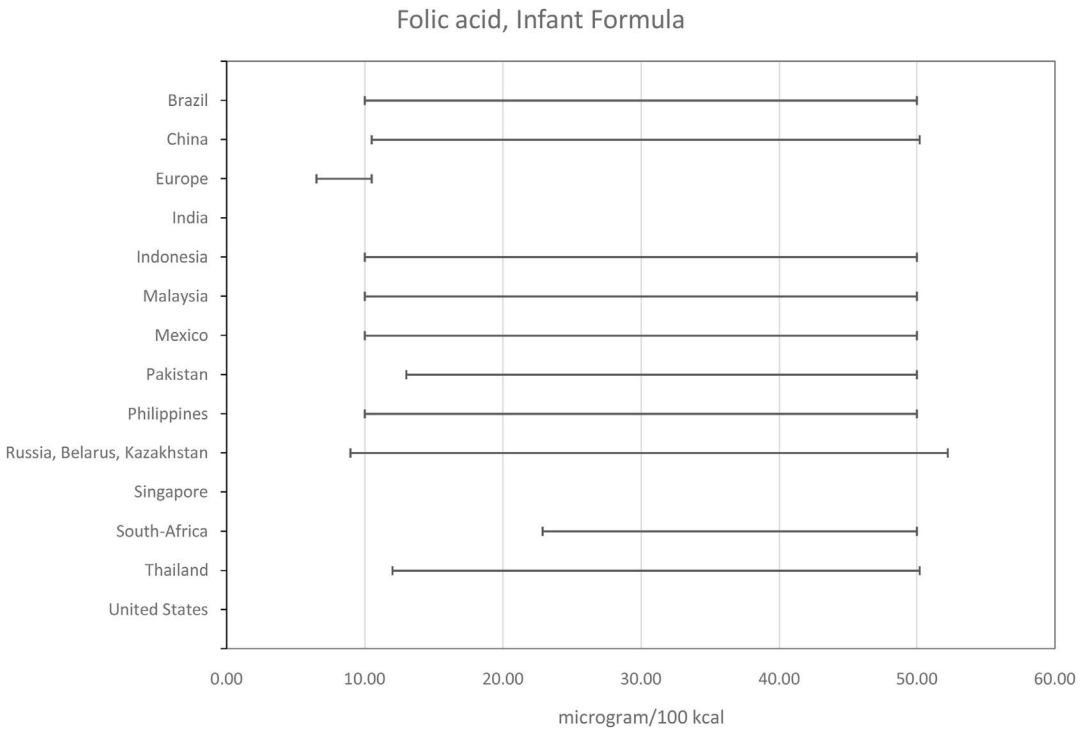

Fig. 6. Product specification range (LSL, USL) for Folic acid in IF and country/region regulatory requirements.

avoid this problem, SPIFAN methods prescribe the preparation of a ready-to-feed mixture from the powdered infant and adult formulas by reconstituting 25 g of powder with 200 g water, followed by taking an aliquot from the reconstituted mixture for analysis. This practice is intended to increase the homogeneity of a sample and represent a standard serving size for the consumer.

Shelf life losses specific to certain vitamins represent another source of variability in addition to general ingredient and analytical variability. This may range from 15% to 75% of the initial value at the end of shelf life, depending on the vitamin, ingredient matrix, and effects of processing and packaging (MacLean et al., 2010). For infant nutrition products that are typically packaged under inert gas, shelf life losses are reduced but nevertheless can still account for 10–15% loss of nutrients (data not published). Considering that the shelf life should be adjusted to avoid losses higher than 20% for instance, it means there are two values that should fit within the specification range, the values

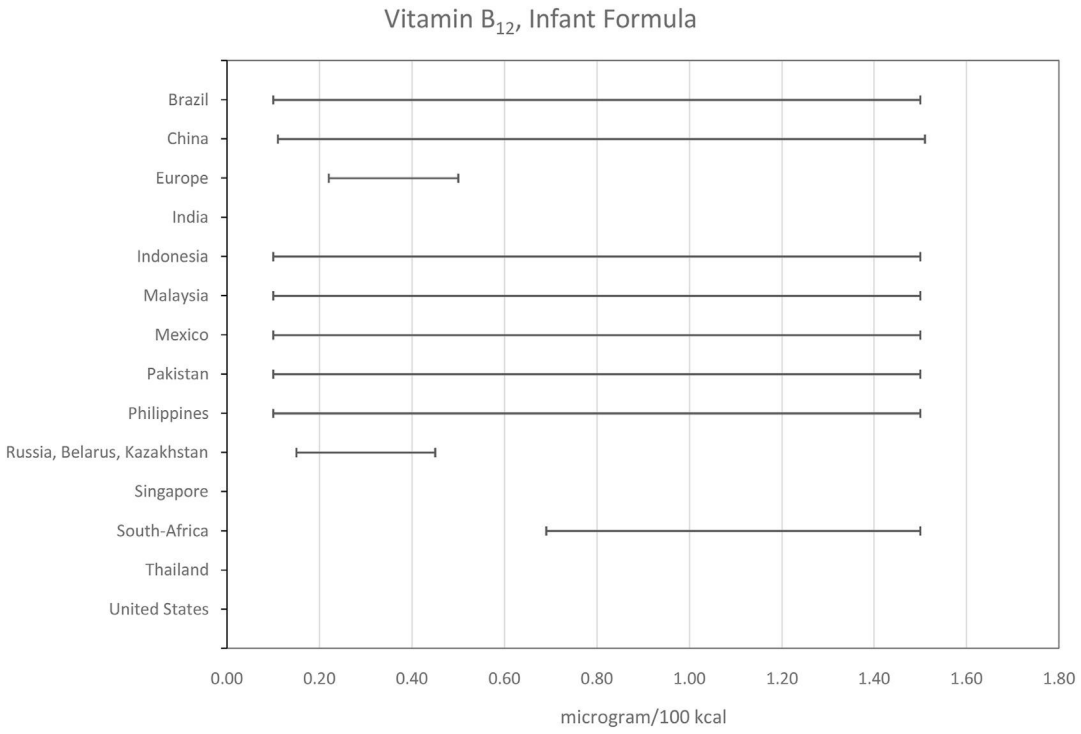

Fig. 7. Product specification range (LSL, USL) for vitamin B_{12} in IF and country/region regulatory requirements.

Table 9
Probability to find a value out of specification range, while manufactured at midpoint of the specification range, when using the SPIFAN methods for nutrients with $C_m < 1$.

	Infant formula					
	Vitamin A %	Vitamin B_1%	Vitamin B_6%	Vitamin B_{12}%	Vitamin D %	Folic acid%
Europe	7.8			1.0	1.7 (18.7[a])	
Pakistan	0.5					
Russia					0.3	
Singapore	1.4					
South Africa	0.6			1.4		0.4
Thailand	1.4					
Follow-up Formula (6-36)						
Europe	7.8			1.0	1.7	
Singapore	1.4					
South Africa	0.6			1.4		0.4
Follow-up Formula young children						
China	1.4					1.0
Europe	0.4			1.0		
South Africa	0.6			1.4		0.4
Foods for Special Medical purposes intended for infants						
Europe	0.4			1.0	1.7	
Russia	7.8	4.8	6.9	2.8		1.0
Singapore	1.4					
South Africa	0.6			1.4		
Thailand	0.6					

[a] Value with new EU Regulation 2019/828 since 14th March 2019.

at the beginning and end of shelf life. Taking the target value in the middle of the largest possible specification range, as done for this paper, allows us to highlight the biggest inconsistencies between the method performance and the regulations. This certainly underestimates the problems from a manufacturer's point of view relative to shelf life losses.

As from Table 3, it can be concluded that the analytical variability (RSD_R) for the SPIFAN methods varies from 3.0 to 15.2% for the concentration range indicated.

In summary, the discussion above confirms that the variability from ingredients and production process contributes to overall process variability, supporting the assumption of an analytical method variance of 1/3 of the overall process variance. This means that the derived target $C_m \geq 1.73$, is most appropriate when assessing compliance of a product with regulatory requirements.

Some of the new SPIFAN methods are not able to deliver results within regulatory limits and tolerances of several countries. Of specific concern are methods for vitamins A, B_{12}, D and folic acid in product categories IF, FUF (6-36), FUF for young children and FSMP, which have the highest number of country/region regulations with problematic C_m. Several country/region regulations for these nutrients show a critical value of $C_m < 1$ including China, some EU member states,

Pakistan, Russia, Singapore, South Africa and Thailand.

The higher the analytical method variability within specific product specifications, the lower the C_m and C_p. The lower the C_p, the more products will fall out of specification range. For the purpose of this paper, we considered a $C_m < 1.73$ undesirable and a $C_m < 1$ critical, because this would cause a $C_p < 1$ and consequently more products out of specifications ($\geq 0.27\%$). Results in this paper show that for the determination of vitamin A in several product categories ($C_m < 1$) there is a maximum probability of 8% of finding an analytical result outside of regulatory requirements due to analytical variability alone. Consequently, such strict regulatory constraints are difficult to enforce even with best-in-class SPIFAN methods.

Since initiating the evaluation of global regulatory requirements for this paper, a new European regulation for vitamin D in IF was adopted by the European Commission on the 14th of March 2019 (EU regulation 2019/828). The Commission amended the Delegated Regulation (EU) 2016/127 to lower the maximum level of vitamin D in IF from 3 μg/100 kcal to 2.5 μg/100 kcal. This lowers the C_m from 0.80 to 0.44, based on Min and Max levels of 2–3 μg/100 kcal. Consequently, for a product manufactured within the regulatory requirements, the probability of finding a test result outside of the regulatory requirements due to the analytical variability alone would be as high as 19%. According to the prescribed approach, with one-third of the variance attributed to the analytical method, a method with an RSD_R of 2.1%, at most, would be required to deliver results which fit within the regulation.

To improve the situation in the future, we encourage continuous efforts toward harmonization of regulatory requirements across global regulatory bodies. However, for the harmonization of regulatory requirements, considerations on risk management approaches are important. For example, the scientific basis for Min and Max levels on vitamin D in IF in the revised CODEX Standard for IF (CXS 72-1981) in 2007 was from a report of an ESPGHAN (The European Society for Pediatric Gastroenterology, Hepatology and Nutrition) expert group (Koletzko et al., 2005). This resulted in a recommendation of vitamin D between 1 and 2.5 μg/100 kcal in IF. Based on EFSA's opinion (EFSA, 2018), the European Commission adopted a range between 2 and 2.5 μg/100 kcal in IF (EU Regulation 2019/828).

Another option to improve the ability to support the determination of compliance with regulatory requirements is the development of analytical methods with less analytical variability. However, as discussed in this paper, current reference methods for IF are already considered state of the art. Selecting new technologies in the future may increase costs of analytical testing as a consequence. In addition, potential new analytical technologies may not be readily available for all countries.

Finally, one other option could be to improve the process and ingredient variabilities to reduce these components on the overall process variability. If the process and ingredient variability are considered to be $< 1/3$ of the total process variance and allow a $C_m > 1.22$, the level at which 2/3 of the variance comes from the analytical method, then we see in this study that for vitamins A, B_{12}, D and folic acid there is still a substantial frequency of country/region regulatory requirements with $C_m < 1.22$. For product categories IF, FUF (6-36), FUF young children and FSMP, there are respectively 13 (24%), 11 (29%), 10 (25%) and 12 (22%) country/region regulatory requirements with $C_m < 1.22$, versus respectively 38 (70%), 23 (61%), 19 (48%) and 37 (67%) when considering $C_m < 1.73$. This means that for these nutrients there is no way to improve the process to compensate the analytical method variability with the current specifications.

5. Conclusion

We have evaluated national regulatory requirements, including Min and Max levels and tolerances from the label declaration for nutrients in IFs, FUFs (6-36) and young children, and FSMPs. The regulatory requirements were compared with the variability of state-of-the-art analytical reference methods to determine the impact of analytical method variability on the assessment of product compliance. Taking the concept of analytical method capability (C_m) as an indicator for regulatory requirements versus the variability of a method, it can be concluded that some of the available reference methods are not fit to assess compliance with the narrow regulatory limits for certain nutrients in several countries. These reference methods have a $C_m < 1$. For a product with a true nutrient value in the middle of the specification range, the probability of finding a test result outside of the regulatory requirements due to analytical variability alone can be as high as 19%. This does not include additional sources of variability related to the production process and raw material composition. Of particular concern are analytical methods for vitamins A, B_{12}, D and folic acid for country/regional regulations or guidelines in China, some EU member states, Pakistan, Russia, Singapore, South Africa and Thailand. This situation could be improved by developing analytical methods with less variability, but this is not a viable option given the excellent precision of SPIFAN methods. Rather, this situation can be improved only by changing and globally harmonizing regulatory limits/tolerances to fit the modern testing capabilities. It should be noted that for the latter, the alignment of approaches to risk management, which are the basis of different regulatory requirements, may be considered. The concept of method capability described in this paper is an adequate tool to help set or evaluate performance requirements of future methods to deliver results which support the assessment of regulatory compliance.

CRediT authorship contribution statement

Erik J.M. Konings: Conceptualization, Methodology, Formal analysis, Writing - original draft. **Antoine Roux:** Methodology, Investigation, Formal analysis, Writing - original draft. **Audrey Reungoat:** Methodology, Investigation, Formal analysis, Writing - original draft. **Nathalie Nicod:** Resources, Writing - original draft. **Esther Campos-Giménez:** Writing - original draft. **Laurent Ameye:** Writing - original draft. **Peter Bucheli:** Writing - original draft. **Sandrine Alloncle:** Writing - original draft. **Julien Dey:** Writing - original draft. **Geneviève Daix:** Writing - review & editing. **Brendon D. Gill:** Writing - review & editing. **Harvey E. Indyk:** Writing - review & editing. **Robert A. Crawford:** Writing - review & editing. **Roger Kissling:** Writing - review & editing. **Stephen E. Holroyd:** Writing - review & editing. **Martine P. van Gool:** Writing - review & editing. **Arnold P. Broek:** Writing - review & editing. **Hans M.M. Cruijsen:** Writing - review & editing. **Dustin E. Starkey:** Writing - review & editing. **Joseph J. Thompson:** Writing - review & editing. **Stefan Ehling:** Writing - review & editing. **Ross Peterson:** Writing - review & editing. **Scott Christiansen:** Writing - review & editing. **Karen Mandy:** Writing - review & editing. **Cristine L. Bradley:** Writing - review & editing. **Shay C. Phillips:** Writing - review & editing. **Julie Moulin:** Conceptualization, Methodology, Formal analysis, Writing - original draft, Supervision.

Declaration of competing interest

None.

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